

CLAIMS:

1. A putative protective antigen against a Mycoplasma, prepared by a method including
- 5 providing
- a sample of a Mycoplasma;
- an antibody probe including at least one antibody against a Mycoplasma produced by a method including;
- 10 providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;
- isolating cells from the biological sample;
- 15 culturing cells in vitro in a suitable culture medium; and
- harvesting antibodies produced from said cells;
- probing the Mycoplasma sample with the antibody probe to detect at least one antigen; and
- isolating the antigen detected.
- 20 2. A putative protective antigen according to claim 1 wherein the Mycoplasma is Mycoplasma hyopneumoniae.
3. A putative protective antigen against Mycoplasma hyopneumoniae, or
- 25 related infections, selected from the group of antigens having approximate molecular weights of 110-114, 90-94, 72-75, 50-64, 52-54 and 46-48 kilodaltons (kD), as herein described, mutants, derivatives and fragments thereof.
4. A putative protective antigen according to claim 3 which is a surface
- 30 protein.

200021 DECEMBER

5. A putative protective antigen according to claim 3 or 4 which is a surface lipo-protein or membrane protein.
6. A putative protective antigen according to any one of claims 3-5 having approximate molecular weight of 110-114, 90-94, 74, 62, 52 and 48 kD.

7. A putative protective antigen according to claim 3 wherein the antigen in the 72-75 kD region contains the following N-terminal amino acid sequence:

AGXLQKNSLLEEVWYLAL

8. A putative protective antigen according to claim 7 further including one or more of the following N-terminal amino acid sequences:

AKNFDFAPSIQGYKKIAHEL

NLKPEQILQLLG

LLKAEXNKXIEZINTXLDN

9. A putative protective antigen according to claim 3 wherein the antigen in the 50-54 kD region contains the following N-terminal amino acid sequence:

MKLAKLLKGFX(N/L)(M/V)IK

ADP(F/I)(R/E)Y(V/A)PQG(Q/A)X(M/N)VG

10. A putative protective antigen according to claim 3 wherein the antigen in the 52-54 kD region contains the following N-terminal amino acid sequence:

AGXWAKETTKEEKS

11. A putative protective antigen according to claim 10 further including one or more of the following N-terminal amino sequences:

AWVTADGTVN

AIVTADGTVNDNKPQWVRKY

12. A putative protective antigen according to claim 3 wherein the antigen in the 46-48 kD region contains the following N-terminal amino acid sequence:

AGXGQTESGSTSDSKPOAETLKHKV

13. A putative protective antigen according to claim 12 further including one or more of the following internal amino acid sequences:

5 TIYKPDKVLGKVAVEVLRVLIAKKNKASR
AEQAITKLKLEGFDTQ
KNSQNKIIDLSPEG

14. An isolated nucleic acid fragment encoding a putative protective antigen against Mycoplasma hyopneumoniae or related infections, said nucleic acid fragment including the following sequence, mutants, derivatives, recombinants and fragments thereof:

	10	20	30	40	50
15	1234567890	1234567890	1234567890	1234567890	1234567890
	ATGAAAAAAA	TGCCACTATA	CCAGAGGAAA	GAGCAGTATA	TAAAATAATT 50
	AAAATTACAT	TTTCTTCATT	TGCGGCGAGAA	TTTTTAAGAA	TTAGTACATT 100
	AAAAAGTAGA	ACAAAAGTTA	TTAATGTAAA	CATTAGCGCA	ATCCTTAAGA 150
20	AAAAATTAAA	AGTTTTATCT	ATTTTTTA	ATCGAAATCC	AACCAGGCAT 200
	AAATCTTTGT	CAGTATTTAT	CAAGTCGGTA	TTTTTCATT	ATTCTACTA 250
	AAATATTATT	TGAATTTGCA	TTTTCCATAA	TCTAAAATTT	TACATTTTTT 300
	TATAACAATT	TTTAAAAATT	ACTCTTTAAT	TTATAGTATT	TTTTTATTTT 350
	TTAGTCTAAA	TTATAAAATT	ATCTTGAATT	TTATTTGAAT	TTTTATAATT 400
25	TAGTACTAAA	AAATACAAAT	ATTTTTTCCT	ATTCTAAGAA	AAATTCATT 450
	TTTAAAAAAA	ATTGATTTTT	ATAGTATAAT	TTGTTTGTAT	AATTGAATTA 500
	ACTTGATTTG	AAAGGGAACA	AAATGAAAAA	AATGCTTAGA	AAAAAATTCT 550
	TGTATTCATC	AGCTATTTAT	GCAACTTCGC	TTGCTCAAT	TATTGCATTT 600
	GTTGCAGCAG	GTTGTGGACA	GACAGAATCA	GGTTCAACTT	CTGATTCTAA 650
30	ACCACAAGCC	GAGACGCTAA	AACATAAAGT	AAGTAATGAT	TCTATTCGAA 700
	TAGCACTAAC	CGATCCGGAT	AATCCTCGAT	GAATTAGTGC	CCAAAAAGAT 750
	ATTATTTCTT	ATGTTGATGA	AACAGAGGCA	GCAACTTCAA	CAATTACAAA 800
	AAACCAGGAT	GCACAAAATA	ACTGACTCAC	TCAGCAAGCT	AATTTAAGCC 850
	CAGCGCCAAA	AGGATTTATT	ATTGCCCTGT	AAAATGGAAG	TGGAGTTGGA 900
35	ACTGCTGTTA	ATACAATTGC	TGATAAAGGA	ATTCCGATTG	TGCCTATGA 950
	TCGACTAATT	ACTGGATCTG	ATAAATATGA	TTGGTATGTT	TCTTTTGATA 1000
	ATGAAAAAGT	TGGTGAATTA	CAAGGTCTTT	CACTTGCTGC	GGGTCTATTA 1050
	GGAAAAAGAG	ATGGTGCTTT	TGATTCAATT	GATCAAATGA	ATGAATATCT 1100
	AAATCACAT	ATGCCCAAG	AGACAATTTT	TTTTTATACA	ATCGCGGGTT 1150
40	CCCAAGATGA	TAATAATTCC	CAATATTTTT	ATAATGGTGC	AATGAAGTA 1200
	CTTAAAGAA	TAATGAAAAA	TTGCGAAAAT	AAATAATTG	ATTTATCTCC 1250
	TGAAGGCGAA	AATGCTGTTT	ATGTCCCAAG	ATGAAATTAT	GGAACTGCCG 1300
	GTCAAAGAAT	CCAATCTTTT	CTAACAAATTA	ACAAAAGATCC	AGCAGGTGGT 1350
	AATAAAATCA	AAGCTGTTGG	TTCAAAACCA	GCTTCTATT	TCAAAGGATT 1400
45	TCTTGCCCCA	AATGATGGAA	TGGCCGAACA	AGCAATCACC	AAATTTAAAC 1450
	TTGAAGGGTT	TGATACCCAA	AAATCTTTG	TAACTCGTCA	AGATTATAAT 1500
	GATAAGGCCA	AAACTTTTAT	CAAAGACGGC	GATCAAAATA	TGACAAATTA 1550

200021-044680

- 29 -

5 TAAACCTGAT AAAGTTTTAG GAAAAGTTGC TGTGAAGTT CTTCGGGTTT 1500
 TAATTGCAAA GAAAAATAAA GCATCTAGAT CAGAAGTCGA AAACGAACTA 1550
 AAAGCAAAAC TACCAAATAT TTCATTTAAA TATGATAATC AAACATATAA 1700
 AGTACAAGGT AAAAATATTA ATACAATTTT AGTAAGTCCA GTAATTGTTA 1750
 CAAAAGCTAA TGTTGATAAT CCTGATGCCT AA 1762

15. An isolated nucleic acid fragment according to claim 14 encoding a putative protective antigen wherein the antigen is in the 46-48 kD region including the following nucleic acid sequence, mutants, derivatives, recombinants and fragments thereof:

	10	20	30	40	50	
	1234567890	1234567890	1234567890	1234567890	1234567890	
15	ATGAAAAAAA	TGCCACTATA	CCAGAGGAAA	GAGCAGTATA	TAAAATAATT	50
	AAAATTACAT	TTTCTTCATT	TGCGCCAGAA	TTTTTAAGAA	TTAGTACATT	100
	AAAAAGTAGA	ACAAAAGTTA	TTAATGTAAA	CATTAGCGCA	ATCCTTAAGA	150
	AAAAATTAAA	AGTTTTATCT	ATTTTTTTTA	ATCGAAATCC	AACCAGGCAT	200
	AAATCTTTGT	CAGTATTTAT	CAAGTCGGTA	TTTTTTCATT	ATTTCTACTA	250
20	AAATATTATT	TGAATTGCA	TTTTCCATAA	TCTAAAATTT	TACATTTTTT	300
	TATAACAATT	TTTAAAAATT	ACTCTTTAAT	TTATAGTATT	TTTTATTTTT	350
	TTAGTCTAAA	TTATAAAATT	ATCTTGAATT	TTATTTGAAT	TTTTATAATT	400
	TAGTACTAAA	AAATACAAAT	ATTTTTTCCT	ATTCTAAGAA	AAATTCATTT	450
	TTTAAAAAAA	ATTGATTTTT	ATAGTATAAT	TTGTTTGTAT	AATTGAATTA	500
25	ACTTGATTTG	AAAGGGAACA	AAATGAAAAA	AATGCTTAGA	AAAAAATTCT	550
	TGTATTCATC	AGCTATTTAT	GCAACTTCGC	TTGCATCAAT	TATTGCATTT	600
	GTTGCAGCAG	GTTGTGGACA	GACAGAAATCA	GGTTCAACTT	CTGATTCTAA	650
	ACCACAAGCC	GAGACGCTAA	AACATAAAGT	AAGTAATGAT	TCTATTCGAA	700
	TAGCACTAAC	CGATCCGGAT	ATCCTCGAT	GAATTAGTGC	CCAAAAAGAT	750
30	ATTATTTCTT	ATGTTGATGA	AACAGAGCCA	GCAACTTCAA	CAATTACAAA	800
	AAACCAGGAT	GCACAAAATA	ACTGACTCAC	TCAGCAAGCT	AATTTAAGCC	850
	CAGCGCCAAA	AGGATTTATT	ATTGCCCTCG	AAAATGGAAG	TGGAGTTGGA	900
	ACTGCTGTTA	ATACAATTGC	TGATAAAGGA	ATTCCGATTG	TTGCCTATGA	950
	TCGACTAATT	ACTGGATCTG	ATAAATATGA	TTGGTATGTT	TCTTTTGATA	1000
35	ATGAAAAAGT	TGGTGAATTA	CAAGGTCTTT	CACTTGCTGC	GGGTCTATTA	1050
	GGAAAAAGAG	ATGGTGCTTT	TGATTCAATT	GATCAAAATGA	ATGAATATCT	1100
	AAAATCACAT	ATGCCCAAG	AGACAATTTT	TTTTATACA	ATCGCGGGTT	1150
	CCCAAGATGA	TAATAATTCC	CAATATTTTT	ATAATGGTGC	AATGAAAGTA	1200
	CTTAAAGAAT	TAATGAAAAA	TTGCAAAAT	AAAAAATTG	ATTTATCTCC	1250
40	TGAAGGCGAA	AATGCTGTTT	ATGTCCAGG	ATGAAATTAT	GGAAGTCCG	1300
	GTCAAAGAAT	CCAATCTTTT	CTAACAATTA	ACAAAGATCC	AGCAGGTGGT	1350
	AATAAAATCA	AAGCTGTTGG	TTCAAAACCA	GCTTCTATT	TCAAAGGATT	1400
	TCTTGCCCCA	AATGATGGAA	TGGCCGAACA	AGCAATCACC	AAATTAAGAC	1450
	TTGAAGGGTT	TGATACCCAA	AAAATCTTTG	TAACTCGTCA	AGATTATAAT	1500
45	GATAAAGCCA	AAACTTTTAT	CAAAGACGGC	GATCAAAATA	TGACAATTTA	1550
	TAAACCTGAT	AAAGTTTTAG	GAAAAGTTGC	TGTGAAGTT	CTTCGGGTTT	1600
	TAATTGCAAA	GAAAAATAAA	GCATCTAGAT	CAGAAGTCGA	AAACGAACTA	1650
	AAAGCAAAAC	TACCAAATAT	TTCATTTAAA	TATGATAATC	AAACATATAA	1700
	AGTACAAGGT	AAAAATATTA	ATACAATTTT	AGTAAGTCCA	GTAATTGTTA	1750
50	CAAAAGCTAA	TGTTGATAAT	CCTGATGCCT	AA		1762

200430-040000

16. A method for producing an antibody against a Mycoplasma including providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;
- 5 isolating cells from the biological sample;
- culturing cells in vitro in a suitable culture medium; and
- harvesting antibodies produced from said cells.
17. A method according to claim 16 wherein the biological sample is taken at a
- 10 predetermined time after the animal has been challenged with a Mycoplasma, preferably 2 to 7 days after challenge.
18. A method according to claim 16 wherein the culturing of cells in vitro further includes addition of helper factors to the culture, said helper factors
- 15 selected from the group including cytokines used alone or in combination, including Interleukin 1, 2, 3, 4, 5, 6, 7 and 8, colony stimulating factors, interferons and any other factors that may be shown to have an enhancing effect on specific B cell secretion.
- 20 19. A method according to any one of claims 16-18 further including a cell activation step including activating the cells isolated to proliferate and secrete and/or release antibodies
- said cell activation step including adding a cell activating agent to the culture medium, said cell activating agent selected from the group including
- 25 mitogens as herein described and helper factors produced by leukocytes, or their synthetic equivalents or combinations thereof.
20. A method according to any one of claims 16-19 wherein the antibody is in the form of the supernatant harvested from the culture medium.
- 30 21. An antibody against a Mycoplasma prepared according to the method of any one of claims 16-20

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22. A method of identifying a putative protective antigen associated with a Mycoplasma, preferably Mycoplasma hyopneumoniae, said method including providing

5 a sample of a Mycoplasma; and
an antibody probe including at least one antibody against a Mycoplasma;

probing the Mycoplasma sample with the antibody probe to detect at least one antigen; and
10 isolating the antigen detected.

23. A method of purifying a putative protective antigen associated with a Mycoplasma, preferably Mycoplasma hyopneumoniae, said method including providing

15 a crude antigen mixture; and
an antibody against a Mycoplasma immobilized on a suitable support;

subjecting the crude antigen mixture to affinity chromatography utilizing the immobilized antibody; and
20 isolating the purified antigen so formed.

24. A method for preparing a synthetic antigenic polypeptide against Mycoplasma, preferably Mycoplasma hyopneumoniae, which method includes providing

25 a cDNA library or genomic library derived from a sample of Mycoplasma; and

an antibody probe including an antibody prepared according to claim 15;

generating synthetic polypeptides from the cDNA library or genomic library;
30 probing the synthetic polypeptides with the antibody probe; and
isolating the synthetic antigenic polypeptide detected thereby.

25. A method according to claim 24 wherein the antibody probe includes an antibody raised against an antigen against Mycoplasma hyopneumoniae, or related infections, selected from the group of antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kilodaltons (kD), as herein described, mutants, derivatives and fragments thereof.

26. A synthetic putative protective antigen in the 72-75 kD region produced by a method according to claim 24 or 25 having an N-terminal amino acid sequence:

AGXLQKNSLLEEVWYLAL

27. A synthetic putative protective antigen according to claim 26 further including internal amino acid sequences:

AKNFDFAPSIQGYKKIAHEL

NLKPEQILQLLG

LLKAEXNKXIEEINTXLON

28. A synthetic putative protective antigen in the 60-64 kD region produced by a method according to claim 24 or 25 having an N-terminal amino acid sequence:

MKLAKLLKGF(N/L)(M/V)IK

ADP(F/I)(R/E)Y(V/A)PQG(Q/A)X(M/N)VG

29. A synthetic putative protective antigen in the 52-54 kD region produced by a method according to claim 24 or 25 having an n-terminal amino acid sequence:

AGXWAKETTKEEKS

30. A synthetic putative protective antigen according to claim 29 further including internal amino acid sequences:

AWWTADGTVN

AIVTADGTVNDNKPNQWVRKY.

31. A synthetic putative protective antigen in the 46-48 kD region produced by a method according to claim 24 or 25 having an N-terminal amino acid sequence:

AGXGQTESGSTSDSKPQAETLKHKV

32. A synthetic putative protective antigen according to claim 31 further including internal amino acid sequences:

5 TIYKPDKVLGKVAVEVLRVLI AKKNKASR
AEQAITKLKLEGFDTQ
KNSQNKIIDLSPEG

10 33. A vaccine or veterinary composition including a prophylactically effective amount of at least one putative protective antigen against a Mycoplasma according to any one of claims 1-13.

15 34. A vaccine or veterinary composition according to claim 33 including a plurality of putative protective antigens selected from antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kilodaltons.

20 35. A vaccine or veterinary composition including an antibody against a Mycoplasma according to claim 21.

35. A diagnostic kit including a diagnostic antigen or fragment thereof according to any one of claims 1-13 and 26-32.

25 37. A method for preventing or treating a Mycoplasma infection, which method including administering to an animal a prophylactically or therapeutically effective amount of at least one putative protective antigen according to any one of claims 1-13.

30 38. An isolated DNA fragment encoding a putative protective antigen against Mycoplasma or related infections, said DNA fragment having a nucleic acid sequence according to Figure 6 or an homologous sequence, and functionally active fragments, mutant, variant or recombinant thereof.

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39. A clone including a DNA fragment according to claim 38.
40. A clone according to claim 39 which is clone pC1-2 as hereinbefore
5 described.
41. An amino acid sequence or functional equivalent thereof encoded by the
DNA fragment according to claim 38.
- 10 42. An amino acid sequence or functional equivalent thereof having the amino
acid sequence of Figure 7
43. A putative protective antigen or antibody substantially as hereinbefore
described with reference to the examples.

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